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Characterization of E. Coli and Total Coliform Organisms Isolated From WI Waters

Characterization of E. coli and Total Coliform Organisms Isolated from Wisconsin Waters and Reassessment of their Public Health Significance

Jon Standridge
Environmental Sciences Section
Wisconsin State Laboratory of Hygiene, University of Wisconsin
465 Henry Mall, Madison, WI 53706

Miel Barman
Environmental Toxicologist
Wisconsin State Laboratory of Hygiene, University of Wisconsin
977 Jonathan Drive
Madison, WI 53713

W.C. Sonzogni
Water Chemistry Program and Environmental Sciences Section,
Wisconsin State Laboratory of Hygiene, University of Wisconsin
465 Henry Mall, Madison, WI 53706

INTRODUCTION

In 1989 the U.S. Environmental Protection Agency promulgated Revised National Primary Drinking Water Regulations pursuant to the federal Safe Drinking Water Act.[1] For Wisconsin, the law drastically increased the number of water systems required to test for microbiological contaminants. The law also introduced the requirement that laboratories not only look for the "total coliforms" group of bacteria, but also the subgroup of fecal coliforms or E. coli. The law assumes a greater public health risk when fecal coliforms or E. coli are found and thus dictates public notification or "boil water orders." The number of microbiological contamination events detected and the frequency of "boil" orders has increased drastically because of the Act.

Concurrent with this increased visibility of microbiological contamination events has come a growing suspicion that we, as public health officials, may be unnecessarily alarming the public when, in fact, there is no real public health threat. This suspicion is fueled by recent reports documenting a number of situations in wells and distribution systems where coliform organisms were growing and multiplying in biofilms yielding positive tests, but where no fecal contamination had actually occurred.[2] Another factor to consider is that the profile of coliform species found in drinking water is very different from the coliform profile of feces.

The literature on coliform differentiation and occurrence both in feces and various water types is very limited. In 1965, Geldreich examined sewage samples and determined that 19-29% of the coliforms in sewage were fecal coliforms.[3] In 1973, Lin tested river waters and sewage treatment plant effluents and determined that fecal coliforms made up, on the average, 17% of the total coliforms found in river water and 8% of those coliforms found in sewage treatment plant effluents.[4]

In 1978, Kinney counted and speciated the coliforms from sewage samples and found E. coli to make up between 13 and 30% of the total coliforms in untreated sewage and between 2 and 11% of the total coliforms in chlorinated effluent.[5] Dufour completed the most thorough study of coliform differentiation in 1977.[6] He states emphatically that E. coli is the only coliform that is an undoubted inhabitant of the gastrointestinal tract. In his tests, 97% of the coliforms isolated from human fecal samples and 94% from animal fecal samples were E. coli. Dufour hypothesizes that contaminated water samples contain disproportionately high levels of non-E. coli coliforms due to infiltration of Klebsiella, Citrobacter and Enterobacter species from non-fecal sources such as run-off, since soil and vegetation often harbour high levels of these three species. He also suggests that these species are more likely to multiply than are E. coli.

The objective of this study is to better characterize coliforms in feces and water using the newly accepted microbiological detection methods. Then, using these characterizations, draw public health conclusions based on total coliform and E. coli data. Specifically, we proposed the following tasks.

1. Determine the total coliform and E. coli populations in 50 fecal samples.
2. Determine total coliform and E. coli populations in sewage and farm runoff samples.
3. Determine the fate over time of total coliforms and E. coli seeded into various water systems at the laboratory bench.

MATERIALS AND METHODS

Fecal specimens were collected without preservatives, refrigerated during transit and tested within eight hours of collection. Approximately 0.1 gm of feces was transferred to 99 ml of phosphate buffered saline using a sterile swab. Subsequent 10x serial dilutions were made from this suspension and tested as if they were water samples using standard protocols.[7] Total coliform levels were determined using the membrane filtration technique plated on m-ENDO agar LES. E. coli was determined using the mTEC membrane filtration technique with the in-situ urease reaction.

The wastewater samples were collected as grab samples directly from the effluent streams of the primary raw sewage clarifiers. The rural runoff samples were collected as grab samples from drainage ditches adjacent to active farming operations during storm water runoff events. Both wastewater and runoff samples were analyzed for total coliforms and E. coli using the standard methods described above.

The environmental fate studies were done on a variety of waters including:

Source	Description	pH	Alkalinity (mg/l)	Hardness mg/l
Madison	1131 ft well	7.4	272	279
DeForest	420 ft well	7.5	272	281
Deerfield	523 ft well	7.4	340	352
Lake Wingra	Lake	8.5	207	268
Lake Monona	Lake	8.7	180	221
Black Earth Creek	Coldwater trout stream	8.6	260	300
Geis well	Private well			
Barman well	Private well			
Synthetic hardwater	Lab prepared	8.4	123	178
Green Bay	treated Lake Michigan			

Water samples (3.5 L) were inoculated with one to 20 ml of a fecal and or sewage suspension prepared as follows. One gm of bovine feces and/or 1 ml of raw sewage was suspended in 100 ml of phosphate buffered saline. The suspension was thoroughly mixed and passed through a #1 Whatman filter to remove the large particulates. Suspensions were prepared from freshly collected sewage or feces and used immediately. Actual feces and sewage were chosen for the inoculum rather than stock cultures of organisms to better represent an actual contamination event. Inoculated waters were held at ambient temperature (19-22°C) in the dark in one gallon polypropylene bottles. The samples were mixed for five minutes (using a magnetic stir bar) immediately prior to daily sample aliquot collection. Aliquots were collected at zero time and once every 24 hours for up to 15 days. Aliquots were tested for total coliform and E. coli using the standard methods described above.

RESULTS AND DISCUSSION

The results of the total coliform and E. coli enumerations from feces are summarized in Table 1. The first column of the table lists the sample source, the second column the total coliform count per gram of feces, the third column lists the E. coli count per gram of feces and the last column lists the percentage of the total coliforms that were E. coli. Overall, 86% of the coliform organisms isolated from various feces samples proved to be E. coli.

In a few cases (sample numbers 15, 27, 31, 38, 43 and 46), the E. coli result exceeded the total coliform count, which is theoretically impossible. This is probably due to the lack of precision that occurs in any coliform enumeration procedure. This is one of the reasons we chose to test a large number of samples. In general, this data set confirms previous work indicating that the predominant coliform in feces is, in fact, E. coli. It also removes doubt related to enumeration techniques used in previous work, since the methods we used are often referred to as the "Gold Standards" to which other methods can be compared.

Table 2 contains the data from samples of raw sewage from three different wastewater treatment plants. Nineteen percent of the coliform population in these samples was E. coli. The data on this table from the three Madison wastewater plant samples is interesting, in that the E. coli and total coliform results are inversely proportional. In a municipal sewage system the fecal contributions from citizens remain fairly stable while water contributions vary substantially from hour to hour and day to day. Therefore, reductions in E. coli are probably due to increases in dilution during high water use periods or from storm water infiltration. Since the total coliform counts go up during these periods, it is reasonable to assume that much of the source of the non-E. coli total coliforms is something other than feces. The other possible explanation of smaller percentages of E. coli in the raw sewage than in feces is that the non-E. coli total coliforms multiply in transit while the E. coli die off. Therefore, the longer the transit time, the lower the percentage of E. coli will be. Using the Madison data from Table 2, low flow rates and the longer transit times they represent demonstrate 28% E. coli while the high flow rates have 4-13% E. coli, just the opposite of what could be explained by regrowth and die-off, lending further credence to the hypothesis that the non-E. coli coliforms are coming from a source other than feces.

Table 3 contains the data from the rural storm water runoff samples. Twenty-nine percent of the total coliform population is made up of E. coli in these samples with a large variation from 4% to 65%. This data set again demonstrate the phenomenon of E. coli percentages being much smaller in environmental samples than in feces. However, there are no clues in the data to suggest a mechanism that might explain the phenomenon.

The data from the environmental fate studies is presented in Tables 4-13. The purpose of these experiments was to study what happens to coliform populations over time in various water types when inoculated with varying amounts of fecal material. In Runs 1 and 2 (Tables 4 and 5) a heavy inoculation (1 gm per liter) of bovine feces and raw sewage was used. This resulted in explosive multiplication of both E. coli and other coliforms probably because of the nutrients provided from the fecal material and sewage.

For the next set of experiments, the feces and sewage was diluted and filtered so that approximately 0.1 gm of fecal/sewage was inoculated into each liter of water. The data in Tables 6 and 7 showed a different trend of coliform and E. coli regrowth using this smaller inoculum. The E. coli numbers dropped off over time while the rest of the total coliform organisms multiplied. The total coliform counts peaked on the fourth day while the E. coli counts dropped off to less than 1% of the original inoculum. The fact that the E. coli counts dropped significantly from 0 to 24 hrs in the Madison tap water is distressing, since water samples submitted for analysis are often 24 hours old when received at the laboratory.

Tables 8-13 represent the next phase of the study using fresh bovine manure where 99% of the coliforms were E. coli. A rapid E. coli die-off was not observed in any of these runs. The 24 hour data in Table 12 appears to be an anomaly and probably represents a lab error.

Table 14 represents a treated municipal water supply that uses Lake Michigan as the source water inoculated with raw sewage. Once again the general trend persists, i.e., the E. coli slowly dies off while the remaining coliforms increase in numbers. Table 15 is the same experiment repeated with a municipal well water supply and shows the same general pattern. Table 16 shows the data from the same experiment repeated using water from a private well demonstrating the same result. The poor precision in the E. coli counts was due to the extremely low numbers encountered on the mTEC plates. The final experiment was performed with raw sewage inoculated into synthetic hard water. Once again, a multiplication of non-E. coli coliforms and a gradual die-off of E. coli and are shown in Table 17.

The survivability of E. coli appears to be unreleated to the water type. For the surface waters, 64% of the E. coli present at time zero was still detectable at 24 hours while 66% were detectable in the well waters (combined data from Tables 6-17). In contrast, the total coliform regrowth phenomenon does appear to depend on water source. For the well waters, total coliforms increased by 32% in the first 24 hours while the surface waters had an overall average decrease of 15%. It is important to point out that there were two occasions where the E. coli population at 24 hours was only 25% of the zero-time counts.

CONCLUSIONS

One can conclude that E. coli is the predominant coliform in feces from humans and a wide variety of animals. Based on enumerations of total coliforms and E. coli using test media designed to be used for detecting these organisms from water. Both sewage and farm run-off have large numbers of total coliforms that are not E. coli, indicating the possibility that coliforms are coming from a source other than feces. The environmental fate studies demonstrate that total coliforms can multiply in water inoculated with sewage or feces. In general, the addition of increasing amounts of sewage or feces onto a sample resulted in increasing multiplication of total coliforms over time, probably due to the nutrients available in the sewage or feces. By contrast, E. coli does not appear to multiply unless huge inoculants are used.

Since much of the non-E. coli coliforms in water appear to be coming from sources other than feces and since these organisms can multiply in a water system, it appears logical to question whether their detection in a water sample has any public health significance. Based on the data from this study, one could easily argue that the detection of total coliforms in the absence of E. coli would almost never represent a fecal contamination event. Their presence does, however, indicate a breach in the water system that needs to be dealt with. But rather than dealing with this breach as a "boil water" public health emergency, it could be handled in much the same way as a main break, i.e., action would be taken immediately to deal with the problem but water users would not need to boil water. E. coli detections would continue to precipitate boil orders and be handled immediately.

The study uncovered an unexpected problem with moving to E. coli as the routine standard. That problem is that E. coli, at least in some of the samples, died off much more quickly than total coliforms. Hence, sample storage and transit becomes a very important issue. Microbiologists have assumed that E. coli stability in water samples over time would be the same as the rest of the coliform group. More work needs to be done in the area of sample preservation. A move to E. coli as the standard for evaluating microbiological stability of water may need to be accompanied by a requirement that samples be stored and shipped to the laboratory on ice.

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Table 1
Comparison of Total Coliform and *E. coli* Concentrations in Fresh Fecal Material

Sample Number	Sample	Total Coliforms Per Gram	*E. coli* Per Gram	% *E. coli*
1	Bovine	4,900,000	3,300,000	67%
2	Bovine	2,700,000	2,700,000	100%
3	Bovine	6,700,000	4,100,000	61%
5	Bovine	500,000	350,000	70%
6	Bovine	540,000	300,000	56%
7	Bovine	520,000	470,000	90%
8	Bovine	3,300,000	3,100,000	94%
9	Bovine	1,700,000	1,600,000	94%
10	Bovine	16,000,000	9,300,000	58%
11	Bovine	38,000,000	17,000,000	45%
12	Cat	24,000	22,000	92%
13	Chicken	1,100,000	700,000	64%
14	Chicken	18,000	10,000	56%
15	Chicken	3,000	6,000	200%
16	Chicken	3,100,000	1,300,000	42%
17	Dog	1,900,000	1,700,000	89%
18	Dog	22,000	13,000	59%
19	Dog	130,000	120,000	92%
20	Dog	9,700	9,300	96%
21	Dog	9,200,000	7,900,000	86%
22	Goat	4,000	3,000	75%
23	Guinea Pig	2,000	1,900	95%
24	Guinea Pig	1,200	1,100	92%
25	Guinea Pig	300	300	100%
26	Human	920,000	610,000	66%
27	Human	250,000,000	380,000,000	152%

28	Human	360,000,000	210,000,000	58%
29	Human	340,000	270,000	79%
30	Human	160,000	150,000	94%
31	Monkey	3,800	9,000	237%
32	Monkey	1,100,000	100,000	9%
33	Monkey	41,000	12,000	29%
34	Mouse	80,000	79,000	99%
35	Mouse	210,000	140,000	67%
36	Mouse	150,000	10,000	7%
37	Pheasant	80,000,000	45,000,000	56%
38	Pheasant	2,800,000	3,700,000	132%
39	Pig	52,000,000	31,000,000	60%
40	Pig	130,000	120,000	92%
41	Pig	12,000,000	5,000,000	42%
42	Rabbit	2,200	2,200	100%
43	Rat	1,700,000	2,600,000	153%
44	Rat	2,100,000	2,300,000	110%
45	Turkey	8,100,000	4,800,000	59%
46	Turkey	1,500,000	1,700,000	113%

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Table 2
Comparison of Total Coliform and *E. coli* Concentrations in Wastewater Treatment Plant Raw Sewage

Sample Site	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
Deerfield	150,000	55,000	37%
Madison	1,200,000	42,000	4%
Madison	380,000	50,000	13%
Madison	470,000	130,000	28%
Verona	13,000,000	1,700,000	13%

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Table 3
Comparison of Total Coliform and *E. coli* Concentrations in Rural Storm Runoff Water

Sample Site	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
Hwy E	250	10	4%
Hwy HV	560	320	57%
Hwy C	1,900	300	16%
Hwy B	5,900	290	5%
Hwy W	230,000	150,000	65%

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Table 4
Water Source - Madison Tap
Fecal Source - Bovine + Municipal Raw Sewage

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	40,000	2,400	6%
24	4,000,000	1,500,000	38%
48	25,000,000	7,000,000	28%
72	11,000,000	5,000,000	45%

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Table 5
Water Source - Lake Monona
Fecal Source - Bovine and Municipal Raw Sewage

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	36,000	2,100	6%
24	4,000,000	640,000	16%
48	51,000,000	7,800,000	15%
72	16,000,000	3,300,000	21%
96	4,800,000	2,300,000	48%
120	1,400,000	540,000	39%
144	2,200,000	NA	NA
168	710,000	320,000	45%

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Table 6
Water Source - Madison Tap
Fecal Source - Bovine and Municipal Raw Sewage

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	6,300	3,500	56%
24	19,000	900	5%
48	20,000	100	1%
72	18,000	20	<1%
96	25,000	20	<1%
120	14,000	40	<1%
144	NA	NA	NA
168	5,900	NA	NA

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Table 7
Water Source - Madison Tap
Fecal Source - Bovine and Municipal Raw Sewage

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	9,800	3,600	37%
24	15,000	3,000	20%
48	4,500	800	18%
72	1,000	1,000	100%
96	570	30	5%
120	180	10	6%
144	250	-	0%
160	90	-	0%

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Table 8
Water Source - Madison Tap
Fecal Source - Bovine

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	1,200	1,200	100%
24	1,200	1,300	108%
48	1,100	920	84%
72	1,100	720	65%
96	540	360	67%
168	110	86	78%
192	70	56	80%
216	74	68	92%
240	56	32	57%
336	6	-	0%

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Table 9
Water Source - Black Earth Creek
Fecal Source - Bovine

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	1,600	1,200	75%
24	1,500	1,100	73%
48	100	870	870%
72	760	520	68%
96	350	210	60%
168	90	50	56%
192	100	56	56%
216	76	48	63%
240	34	36	106%
336	16	16	100%

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Table 10
Water Source - Madison Tap
Fecal Source - Bovine

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	1,550	1,460	94%
24	1,370	1,070	78%
48	1,200	1,190	99%
72	910	870	96%
96	1,250	1,020	82%
120	960	720	75%
144	940	860	91%
172	670	620	93%
196	10	NA	NA

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Table 11
Water Source - Black Earth Creek
Fecal Source - Bovine

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	1500	1300	87%
24	710	620	87%
48	290	180	62%
72	110	93	85%
96	540	430	80%
120	300	260	87%
144	210	180	86%
172	220	150	68%
196	78	76	97%

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Table 12
Water Source - Barman Well
Fecal Source - Bovine Feces

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	1,600	1,300	81%
24	780	280	36%
48	1,900	1,000	53%
72	1,900	1,000	53%
96	1,800	500	28%
120	2,600	940	36%
144	3,200	940	29%
160	2,700	830	31%
184	3,300	870	26%

Water Source - J. Barman Well
Fecal Source - Bovine Species

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	1,700	1,500	88%
24	1,200	1,000	83%
48	900	810	90%
72	580	670	116%
96	560	910	163%
120	560	270	48%
144	350	210	60%
160	290	420	145%
184	60	120	200%

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Table 15
Water Source - DeForest Tap
Fecal Source - Raw Sewage

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	4,000	1,900	48%
24	6,700	1,500	22%
48	9,400	1,800	19%
72	5,400	1,500	28%
96	11,000	1,500	14%
120	11,000	900	8%

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Table 16
Water Source - Geis Well
Fecal Source - Raw Sewage

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	4,300	160	4%
24	2,000	40	2%
48	1,300	80	6%
72	780	240	31%
96	660	60	9%
120	740	-	0%
144	200	-	0%

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Table 17
Water Source - Synthetic Hard Water
Feces Source - Raw Sewage

Time In Hours	Total Coliforms Per 100 mls	*E. coli* Per 100 mls	% *E. coli*
0	5,500	680	12%
24	7,900	400	5%
48	5,700	200	4%
72	1,700	160	9%
96	1,200	120	10%
120	2,000	60	3%
144	1,900	20	1%
168	1,000	20	2%

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Characterization of E. Coli and
Total Coliform Organisms
Isolated From WI Waters